A ROLE OF TRPV CHANNELS IN THE DEVELOPMENT OF NEUROPATHOLOGICAL SYNDROMES

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Olena P. Kostyuk *was born on29 January 1957 in Kyiv (Ukraine). In 1980 graduated from Kyiv Medical Institute. 1980-1983: post-graduate at Komissarenko Institute of Metabolism and Endocrinology, Ukrainian Academy of Medical Sciences, after which she was invited to diabetological department at the same institute. In 1986 defended dissertation on the item "Diabetes Mellitus and macroangiopathy" in which underlined the main features of Juvenile Diabetes Mellitus and received Ph.D. In 1999 produced dissertation on the item "Changes of calcium homeostasis in nervous cells during development of diabetic neuropathy" and received Dr. in Medical Science. In 2001 moved to the Department of general physiology of nervous system at the Bogomoletz Institute of Physiology (Kyiv). Investigation directions: Diabetologia (hormonal changes under different types of diabetic mellitus; changes in the intracellular calcium homeostasis and calcium currents under the condition of experimental diabetes, pain neuropathy, Alzheimer disease, TRP channels). Used the radioimmune technique for hormonal research and fluorescent indicators technique for investigation of changes in pathogenesis of diabetes mellitus and of intracellular calcium in different types of neurons. Kostyuk E.P. is an author of 110 scientific papers and co-author of three books, one of which was awarded Bogomoletz revered prize (2005, NAS Ukraine). Member of the professional section of the American Diabetes Association from June 2001. The main publications are in the international journals: Diabetologia, Neuroscience, Clinical Diabetologia, NeuroReport, Fiziology Journal (UKR), Neurophysiologiya/ Neurophysiology (UKR).*

Calcium signaling is the most important mechanism responsible for the function of exactable cells. Its kinetic characteristics determine the transmission of excitation across neuronal networks and its changes in pathological conditions.

One of the problems with which the group dealt was the investigation of calcium homeostasis during the development of diabetic pain neuropathy. Type 1 of diabetes mellitus is tightly related to this problem. Its main syndromes are acute

pain, hyperalgesia, analgesia and probably hypoalgesia connected with the prolongation of the development of diabetes mellitus. The main mechanisms of development of diabetic neuropathy are alterations in glycolis, development of sorbitol pathway and aldose reductase. They also include two-way changes in the distribution of different ions including Na^+, $K^{\cdot+}$, Cl^-. At the same time, the role of Ca^{2+} in pain syndrome is still not completely clarified.

All our investigations were carried out on small and medium size nociceptive neurons of dorsal root ganglia connected to the C and Aλ fibers which differ in the action potential transmission and translation of pain signals. Changes in the function of intracellular structures of neurons including mitochondria, endoplasmic reticulum and TRPV channels participate in transductions of pain signaling (Kostyuk et al., 1998, Kruglikov et al., 2001).

It was found that during development of diabetic neuropathy the most changes occur at the decay phase of calcium transient. For instance, it was recorded that mitochondria begin to couple less Ca^{2+} inside by changes in the function of calcium uniporter and diminution of cytosol Ca^{2+} becomes smaller (Kostyuk et al., 2004). At the same time, we recorded the prolonged return of the cytosolic calcium to the basal level, which is possibly linked with the changes in $Na^+/Ca^{\cdot 2+}$ exchanger function (Shishkin et al., 2002) The changes in the function of calcium uniporter may be connected with the changes in mitochondrial membrane proton gradient. These processes may lead to the reduction of ATP formation, which will be connected with the diminution of SERCA production. Besides, it is possible that character of changes in the neurons of dorsal root ganglia and spinal cord may be different as we found that mitochondria and endoplasmic reticulum are differently localed in the peripheral and central systems: in the dorsal root ganglia mitochondria are localized near the membrane, whereas in spinal cord - near the center of neurons. Also, the changes in synaptic transmission of glutamate are possible. A great role may be played by different pain neuromodulators. Further the pain signal is transmitted by spino-thalamic tract neurons (Shutov et al., 2002).

In the case of caffeine application after depolarization of the cell, caffeine induces a significant overshoot of cytosolic calcium. With the help of using caffeine in calcium- containing and calcium-free solutions, it was shown that caffeine transients are substantially change in the solution with calcium. Due to our data for preapplication of CCCP, the caffeine induced transients become slower, while in the case of application of CCCP to the caffeine decay phase, the level of Ca^{2+} in cytosol did not change substantially. This indicates that mitochondria and endoplasmic reticulum are tightly connected by calcium exchanger mechanisms. In the case of diabetes mellitus, the reverse of calcium to the basal level became substantially prolonged (Stepanova et al., 2004, Kostyuk, 2007).

Probably, this change in the function and interaction of intracellular structures is one of the reasons for changing the pain signal transduction during neu-

ropathy. Another reason may be changes in T-type currents discovered by our team (Pinchenko et al, 2002, Kostyuk et al, 2003). Also, changes in synaptic transmission by glutamate are possible (Kostyuk, 2001).

We also investigated the characteristics of TRPV channels and the problem of their connection with pain transduction. TRPV channels are a subgroup of the large group of TRP channels. We put our attention *on* the subgroup of the TRPV1 channels (so called capsaicin receptors as far they are sensitive to the pain evoking substance isolated from chilly pepper) in the case of inflammation and neuropathy, high temperatures, and influence of pH. The availability of pain neuropathy was investigated with the Von Frey method and forskolin probes. It must be taken into account that till today the question whether they are store operated channels on which we tried to give the answer.

A group TRPV1 receptors was found on the membranes of nociceptive neurons and inositol-three-phosphate endoplasmic reticulum. During our investigations by application of capsaicin we established that in all types of nociceptive (small and medium) dorsal root ganglion neurons we observed strong elevation of calcium transients. This response was greater than that which occurs after depolarization of the cells. In large (proprioceptive) neurons such changes were not fixed. In our experiments, we have observed significant differences in capsaicin mediated responses between small-sized and medium-sized nociceptive neurons (Stepanova, 2005). When application of capsaicin was eliminated by preapplication of TRPV1 selective blocker, namely capsazepin, which confirms exclusive involvement of TRPV1 as calcium channels connected with pain transduction, this effect disappeared.

Such changes in the calcium level in the cytosol can be one of the mechanisms that modified the translation of pain signals in the cells, probably by calcium oscillation due to changes in the function of mitochondria in the case of pain neuropathy. These changes may be also a result of different expression of TRPV1 in corresponding types of nociceptive neurons. It is very notable that cell responses to capsaicin application after previous depolarization were significantly changed as compared to those without it. It was also found that TRPV1 channels during short time of capsaicin application are characterized by large response with prolonged calcium transient (about 300 s). Furthermore, the answers to capsaicin application were not similar at different durations of such application. Changes occur with prolongation of the delay between depolarization and following capsaicin application (3, 7, and 10 s correspondingly), but accurate time dependence curve was not observed. Probably, the response to capsaicin application and transduction of pain by them are not time dependent. At the same time, such a response to the capsaicin application became greater with time. This can lead to greater hyperalgesia or to hypoalgesia with time. The reason for it may be changes in the influence of bradykinin or anadamide of membrane potential sensors in the case of inflammation or the inositol-three

phosphate receptors or others (Romanenko et al, 2009). Our estimation of calcium influx through TRPV1 pore with respective driving force through plasma membrane for all types of the delay can be described by linear approximation of influx-driving force dependence which does not show easily observable positive tendency but rather vise versa.

It is possible that changes from hyperalgesia and analgesia may be connected with prolongation of naturopathic development. This can be due to changes in expression of capsaicin sensitive receptors on nociceptive neurons or their desensitization because of the changes in their phosphorylation. Besides this, in capsaicin action participate the effects triggered by T type calcium channels (which react to temperature), cation-nonselective nucleotides, and N-type high voltage calcium channels.

Finally, it should be mentioned that these receptors were also found in the structures of central nervous system: on hypothalamus, limbic structures, and hippocampus. So, they may change synaptic plasticity and work as low-cold and high-temperature sensors, but this question is still under investigation.

An interesting result was obtained by searching pharmacological modulators of calcium signaling in nociceptive neurons. Application of gabapentin (an analog of GABA), which is usually used as an antiepileptic substance, exerted an inhibitory effect only on medium-sited neurons responsible for transmission of pain signals. Calcium transients were depressed by 37% in capsaicin–sensitive neurons indicating the connection of gabapentin action with some specific calcium channels in these neurons.

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